NEPHROTIC GLOMERULONEPHRITIS*

DAVID B. JONES, M.D.
From the State University of New York, Upstate Medical Center, Syracuse 10, N.Y.

It has been apparent for many years that glomerulonephritis may present a pleomorphic clinical picture. On the basis of clinical and laboratory findings, the disease has been separated into two types: nephritic glomerulonephritis (Ellis type I) and nephrotic glomerulonephritis (Ellis type II).[1] Nephritic glomerulonephritis is characterized by a history of streptococcal infection (Lancefield group A, usually type 12),[2] a characteristic latent period, gross hematuria, hypertension, slight edema, oliguria, albuminuria, elevated antistreptolysin o titers, and a good prognosis (85 to 90 per cent recovery). Nephrotic glomerulonephritis, on the other hand, is delineated by an absence of any preceding history of bacterial infection, normal antistreptolysin titers, insidious onset of marked edema, albuminuria without significant hematuria, ascites, hyperlipemia, normal blood pressure, absence of azotemia, and a poor prognosis. From the differences enumerated above it is not surprising that many investigators believe that these types of glomerulonephritis are distinct diseases.[1] Clinically, it may be impossible to separate them from each other and from amyloidosis, diabetic glomerulosclerosis, chronic pyelonephritis, and nephrosclerosis.[3] However, these diseases generally are distinguishable pathologically.

Certain cases of nephrotic glomerulonephritis (nephrosis) in children in which hematuria, hypertension, and azotemia are absent have been called pure lipid nephrosis. Many of these children eventually develop hematuria, azotemia, or hypertension and are said by the clinicians to show a transition from a nephrotic to a nephritic picture. Inasmuch as these so-called nephritic patients will show pathologically only evidence of nephrotic glomerulonephritis, it appears that the different manifestations of these cases are all part of the natural evolution of nephrotic glomerulonephritis.

The nature of the microscopic changes in nephrotic glomerulonephritis have been controversial for years. When death occurs early in the course of this disease from intercurrent infection, the structural glomerular changes may be so minimal that some investigators have claimed that no glomerular lesion exists and that the tubular

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lesions indicate a primary tubular disease. While the lesions of the glomeruli may be so minimal that they are overlooked, a small number of definitely scarred glomeruli usually are present.

With progressive activity of the disease, the glomerular changes become more apparent and thus well defined glomerular lesions usually are seen in the older child and adult with nephrosis. Bell[4] described thickened basement membranes about the glomerular capillaries in the nephrosis of adults and noted a suggestion of vacuolization in these membranes. He designated this lesion as membranous glomerulonephritis. McManus[5] noted vacuolization in the mesangium of the glomerulus in nephrosis and demonstrated neutral fat in the vacuoles.

In addition to the membranous lesion of nephrotic glomerulonephritis, Ellis[1] described a peculiar nodular glomerular scar. It was characterized by an enlarged glomerulus with hyaline scar tissue in the center of each lobule of the tuft surrounded by thick-walled capillaries. This lesion may be mistaken for diabetic glomerulosclerosis. Bell[4] called this lesion chronic glomerulonephritis with the clinical picture of lipid nephrosis of the mixed type. Allen[6] uses the term, chronic lobular glomerulonephritis.

The finer structure of the glomeruli of nephrotic glomerulonephritis is not well known. The present study is an attempt to use newer techniques to elucidate the different morphologic forms of this disease.

Methods

The basic histologic procedure of this study was the use of the periodic acid silver methenamine sequence which reveals the fine structures of the glomerular connective tissue in health and disease better than any other technique. It stains essentially the same structures as the periodic acid-Schiff (PAS) stain but with far greater contrast. This permits the resolution of delicate structures which would be missed by the PAS stain. The use of a gold toning procedure seems to exaggerate the contrast even more. It must be stressed that thin sections, 1 to 3 μ thick, are a prerequisite for the visualization of fine details in the glomerulus.

Periodic Acid Silver Methenamine Technique

Formalin, Carnoy's, and Bouin's fixatives give satisfactory results, but fixation in Zenker's or osmic acid solutions is not adequate for this procedure. Due to the high intensity of the stain, sections should be no thicker than 1 to 3 μ.

Procedure

Hydrate sections to distilled water containing 0.5% periodic acid at room temperature for 15 minutes

Wash well in distilled water

Place in Gomori's silver methenamine solution at 45° to 50° C. for 1½ to 3 hours (Gomori's stock solution contained 100 cc. of 3% methenamine and 5 cc. of 5% silver nitrate); add 6 cc. of 5% borax per 50 cc. of stock solution

Wash in distilled water
Tone in 0.2% gold chloride solution for 2 minutes
Wash in distilled water
Place in 3% sodium thiosulfate for 2 minutes
Wash well in tap water
Counterstain with hematoxylin and eosin, or any other suitable stain

Sections may be removed from the silver bath at any time, rinsed in distilled water, and examined under the microscope. If further stain is needed, they may be rinsed in distilled water and replaced in the silver bath. If the slides are overstained, an extremely dilute solution of potassium ferricyanide may be used to destain them to the desired intensity.

Counterstaining with acid dyes is progressively inhibited by the silver stain in much the same way as with osmic acid. Nuclear staining by the silver is seen with prolonged exposure to the silver methenamine solution; hydrolysis of nucleic acids occurs and a silver Feulgen reaction results.

This study is based on the examination of the kidneys of 20 cases of nephrotic glomerulonephritis which were divided into four groups: 6 cases presented minimal glomerular lesions; 6, moderate glomerular lesions; 3, the lesion of chronic lobular glomerulonephritis; and 5, chronic membranous glomerulonephritis.

Results and Discussion

The normal glomerulus in this discussion is considered to be a tuft of capillaries invested by the glomerular epithelium and the prominent subepithelial basement membrane. This basement membrane is continuous with the basement membrane of Bowman's capsule and the proximal convoluted tubules. The capillaries of the tuft are covered by this basement membrane in the same manner as the serosa covers the small bowel.[7-14] A mesentery-like structure thus formed may be called the glomerular stalk or mesangium. Two types of cells are present within the subepithelial basement membrane. Morphologically, there is little difference between them but they may be distinguished by their location and by what they do. The endothelial cell lies flattened against the basement membrane and it forms the endothelial tube of the vessel. The mesangial connective tissue cell lies in the mesangial stalk and it is surrounded by silver-positive fibrillar tissue which is of an irregular spongy nature and stains like the material of the basement membrane. Since this fibrillar material has qualities which are different from other fibrillary connective tissue, the term fibromucin is considered appropriate.

With disease, the endothelium of the capillaries may be separated from the overlying basement membrane by edema, exudate, or other material. Very soon another basement membrane becomes visible adjacent to the endothelium; thus a pericapillary connective tissue space

bounded by basement membranes is formed. Although electron microscopists cannot clearly demonstrate an endothelial basement membrane,[14] the fact that it rapidly becomes visible in disease suggests that normally a thin basement membrane of at least molecular thickness must exist. Aterman[15] and Gersh and Catchpole[16] have shown that basement membranes may thicken rapidly in disease.

The changes in inflammation of connective tissue are predominately the result of exudation and reactions by the fibroblasts. In the glomerulus the same principle holds true. Changes in the glomerular connective tissue other than exudation would appear to be the result of metabolic changes in the connective tissue cells (mesangial cells) so that they lay down more basement membrane substance, fibromucin, or hyalin. The endothelial cells appear to be of little significance since they are prevented from budding by the intact basement membrane. What is the nature of the tissue in the glomerular scars? It is not collagen, reticulin, or elastin, as can be demonstrated by histologic and electron microscopic techniques. It is composed of two materials: hyalin, an eosinophilic material identical to that seen in sclerotic arterioles, and fibromucin, which stains the same as basement membranes. With the periodic acid silver methenamine stain, hyalin is silver-negative but fibromucin and basement membranes are silver-positive.

Nephrotic Glomerulonephritis in Children

In the nephrotic child who dies of intercurrent infection relatively early in the course of the disease, changes in the structure of the glomeruli are minimal (Fig. 1). At the most, a slight increase in the number of mesangial connective tissue cells may be seen (Fig. 2). The tubules, on the other hand, may show striking changes with the accumulation of hyaline droplets and fatty vacuoles in the proximal convoluted segments.

The significance of the above cases is not that there is little glomerular change in nephrotic glomerulonephritis but that many of these children die before well defined lesions have developed. In those instances in which certain authors claim that there are no glomerular lesions in lipid nephrosis, we feel sure that their cases were either milder or earlier stages of the disease.

Some children die with azotemia complicating their nephrotic picture following persistent active disease. In these patients we see definite evidence of the destructive nature of the process on the glomeruli. An increase in connective tissue in the center of each

glomerular lobule and some variable thickening of pericapillary basement membranes may be seen (Figs. 3, 4, and 5). The lumina of some of the capillaries may be partially blocked by lipid-filled cells (Fig. 6). At this stage tubular atrophy and dilatation is very evident and often appears to be out of proportion to the degree of glomerular change.

Nephrotic Glomerulonephritis in Older Children and Adults

Older children and adults do not succumb as frequently to fatal intercurrent infections and thus tend to die of their disease. The increased maturity of their defense mechanisms may be the answer for this. The more advanced glomerular lesions are of three general types: the moderate scar of late nephrosis of childhood, the exaggerated form of this lesion in adults (chronic lobular glomerulonephritis), and the chronic membranous lesion.

The kidney of chronic lobular and chronic membranous glomerulonephritis may either be small or near normal size depending on the degree of tubular atrophy. The kidney with moderately scarred glomeruli usually is near normal size since tubular atrophy generally is slight in this lesion. Fatty and hyaline droplets may be found in the tubules. Interstitial fibrosis and arteriosclerosis tend to be proportional to the degree of tubular atrophy. In chronic nephritic glomerulonephritis the old glomerular scars have been removed, so that only a fraction of the original number of glomeruli remain. With the periodic acid silver methenamine stain, various stages of resorption of the glomerular scars of chronic nephritic glomerulonephritis may be demonstrated. Even in the shrunken fibrotic kidneys of chronic nephrotic glomerulonephritis an approximately normal number of scarred glomeruli may be seen.

Chronic lobular glomerulonephritis is characterized by large glomeruli, each lobule of which is distended by a central mass of cellular scar tissue (Fig. 7). The individual lobules tend to be club-shaped and a few residual thick-walled capillaries lie at the edge of the lobules. The central connective tissue scar (fibromucin and hyalin) is laid down in the region of the mesangium and as it enlarges it presses the capillaries to the periphery (Fig. 8). The earlier stages of this process may be seen in cases showing the moderate glomerular scars in which the scarring of each lobule has just started (Fig. 4). In some of the far advanced cases of chronic lobular glomerulonephritis silver-negative hyalin is deposited between the fibers of the connective tissue

(fibromucin) of the central scars and between the two layers of basement membrane about the capillaries. This may become so extreme that little silver-positive basement membrane substance remains in the glomerulus. In far advanced lesions few patent capillaries remain in the glomeruli. Early in the evolution of this lesion, the moderate scar, double contoured, thin basement membranes surround the capillaries; i.e., an epithelial basement membrane and a thickened endothelial basement membrane separated by connective tissue fluid are visible. Later more glomerular connective tissue is laid down (fibromucin) so that the capillaries are surrounded by dense scar. Very late in the evolution of this lesion the capillaries are obliterated and only nodular masses of scar tissue remain.

To the beginner, the lesion of chronic lobular glomerulonephritis looks like that of diabetic glomerulosclerosis. The absence of extensive hyaline arteriolar sclerosis of the afferent arteriole, the well developed cellularity of the lobular scars, the uniform involvement of all glomeruli, and the evidence of marked capillary obliteration clearly distinguish this lesion from that seen in diabetes mellitus.

The membranous form of nephrotic glomerulonephritis shows a characteristic red hyaline thickening of the basement membranes about the capillaries of the glomeruli with the hematoxylin and phloxine stain (Fig. 9). The glomeruli show some increased cellularity and often the capillary lumina are narrowed by the thickened walls. In rare glomeruli an epithelial crescent may be present. The glomerular basement membrane is thickened from two to four times. In describing this change, Bell[4] noted a vacuolated appearance of the membranes. In the present investigation it has become apparent that this lesion is the result of a peculiar modification of the epithelial basement membrane of the glomerulus. With the periodic acid silver methenamine stain this thickened membrane resolves itself into a much less thick epithelial membrane from which protrudes externally innumerable short silver-positive projections of club or mushroom shape. Between these projections is a silver-negative hyaline material which is in a droplet form (Fig. 10). This hyalin may be differentiated from overlying epithelial cytoplasm by Masson's trichrome stain in which the hyalin stains green and the cytoplasm rose. The PAS stain shows the same structure but there is insufficient contrast to make the lesion clear. In certain early lesions it appears that the hyaline droplets accumulate within the epithelial basement membrane. As they enlarge they push peripherally. When they are sufficiently large the external layer of basement membrane is no longer visible and the hyaline drop-

lets appear to be separated from the structure by silver-positive clubs. This hyalinosis of the basement membrane may become so extreme that little silver-positive material remains (Fig. 11). This alteration of the basement membrane is seen both in the chronic lobular and the chronic membranous lesions. The significance of the hyalin is conjectural. It appears likely that hyalin is an abnormal connective tissue deposit resulting from chronic injury to the cells of the mesangium which are responsible for maintaining the connective tissue spaces and basement membranes of the glomerulus.

In summary, the lesions of nephrotic glomerulonephritis may be divided into the minimal lesion of childhood in which death is usually due to intercurrent disease, the moderate lesion of children and young adults who die of renal failure, the chronic lobular lesions, and the membranous lesions of adults. An explanation as to why some adults develop the chronic lobular lesion and others the membranous lesion is not available.

The Biologic Relationship of Acute and Chronic Glomerulonephritis (Nephritic Glomerulonephritis, Ellis Type I) and Nephrotic Glomerulonephritis (Ellis Type II)

A large number of differences, epidemiologic, clinical, and morphologic, exist between nephritic and nephrotic glomerulonephritis. In spite of these differences there is evidence to suggest that they belong to one family.

It has been shown that both nephritic and nephrotic patients possess circulating antiglomerular auto-antibodies. The antibody titers rise with activity and fall following remission,[17] and the reaction is complement binding.[18] This fact correlates with the known depression of blood complement in the acute phases of the diseases.[19] It is further substantiated by the demonstration that human gamma globulin is bound to glomeruli in both nephrotic and nephritic glomeruli, the gamma globulin being an antiglomerular auto-antibody.[20]

With acute diffuse glomerulonephritis it appears that a streptococcal (Lancefield group A, type 12) infection usually triggers the auto-antibody production. It is postulated that a streptococcal product combines with the glomerular connective tissue to form an antigen which results in antibody formation. Even though glomerular connective tissue is not in itself antigenic, it combines like a haptene with the antibody and injury to the glomerulus of the Arthus type results. After a variable time the antibody falls to unreacting levels and the

injurious action ceases. Healing or scarring depends mainly on the severity and the duration of the injury. In most cases resolution is almost complete.

In nephrotic glomerulonephritis no triggering mechanism has been definitely established. The process is often gradual in onset and shows remissions and exacerbations. An auto-antibody formation similar to that of acute glomerulonephritis appears to be present but its mechanism is still unknown.

The Natural History of Nephrotic Glomerulonephritis

As a result of the high rate of death of nephrotic children from bacterial infection, the long-term effect of the disease on renal function is difficult to evaluate. In the edematous phase, hypoproteinemia, hypogammaglobulinemia, and other non-specific factors result in a high incidence of pneumococcal or streptococcal infections. Infection plus a few cases of gross electrolyte disturbance or heart failure result in about a 50 per cent rate of death in this phase of the disease. With the advent of antibiotics this incidence of death from infection will decrease so that a larger proportion of nephrotic children will live out the disease process.

Rennie,[21] in a group of 29 cases of nephrotic glomerulonephritis, found only 3 in which apparently complete healing had occurred after 7 to 15 years. Davson and Platt[3] reported 8 of 34 patients with this disease alive and well but 3 of the 8 patients showed albuminuria. Schwarz and associates[22] found only 4 of 40 nephrotic patients completely healed long after the onset of their disease. These figures indicate that in many patients progressive glomerular injury results in uremia. It is unfortunate that the figures of survival are not expressed in 5, 10, and 20-year periods as for cancer. It is impossible at the present time to estimate the relative prognosis of a patient in various phases of this disease.

With the advent of hormone, antibiotic, and other forms of therapy a larger proportion of patients survive the edematous phase of the disease but albuminuria often persists. We can hope that with this therapy a smaller percentage will develop azotemia or hypertension.

Summary

On clinical, pathologic, and natural historical grounds, nephrotic glomerulonephritis and nephritic glomerulonephritis have similarities and differences. It appears that a peculiar auto-antibody injury is

present in both processes but the trigger mechanism has not been discovered for nephrotic glomerulonephritis.

Pathologically, the types of glomerular lesions seen in nephrotic glomerulonephritis have been divided into the minimal lesion of childhood, the moderate lesion of the uremic child or young adult, the chronic lobular lesion, and the membranous lesion. Certain new observations of these lesions have been discussed.

It is hoped that future presentations of clinical material on nephrotic glomerulonephritis will make use of 5, 10, and 20-year survival statistics so that a better concept of ultimate prognosis may be reached.

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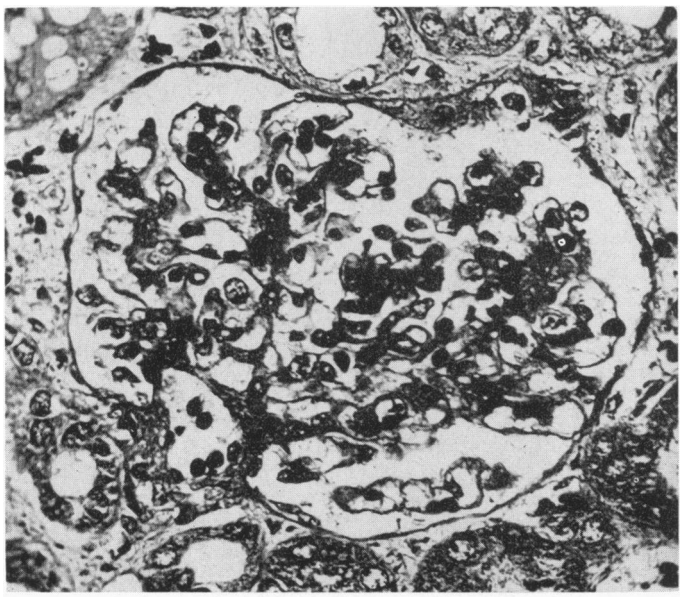
LEGENDS FOR FIGURES

FIG. 1. Nephrotic glomerulonephritis; minimal lesion in a child with lipid nephrosis. Hematoxylin and phloxine stain. × 385.

FIG. 2. Nephrotic glomerulonephritis; minimal lesion in a child with lipid nephrosis. The prominent dark-staining mesangial material filled with nuclei is the outstanding glomerular lesion. Silver methenamine, hematoxylin and eosin stains. × 1,000.

FIG. 3. Nephrotic glomerulonephritis; moderate lesion. There is an accumulation of fibromucin in the center of each lobule. Hematoxylin and phloxine stain. × 345.



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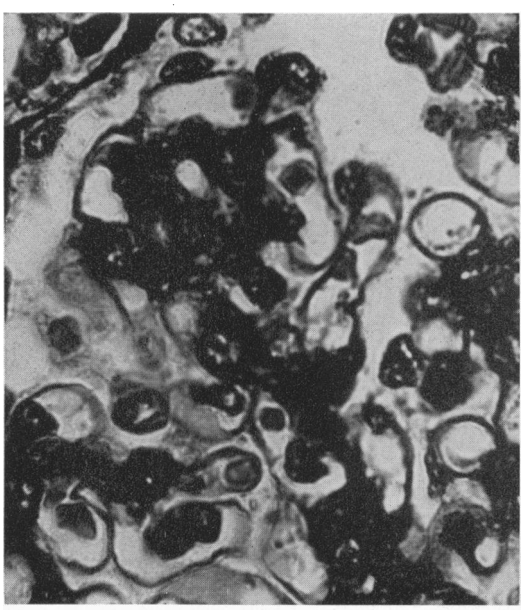

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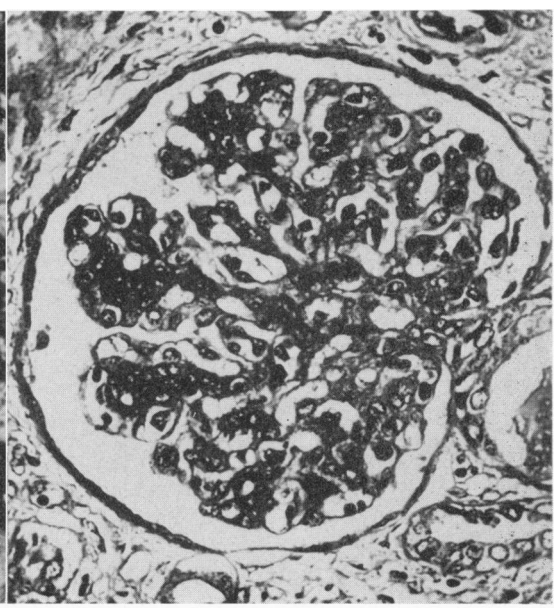

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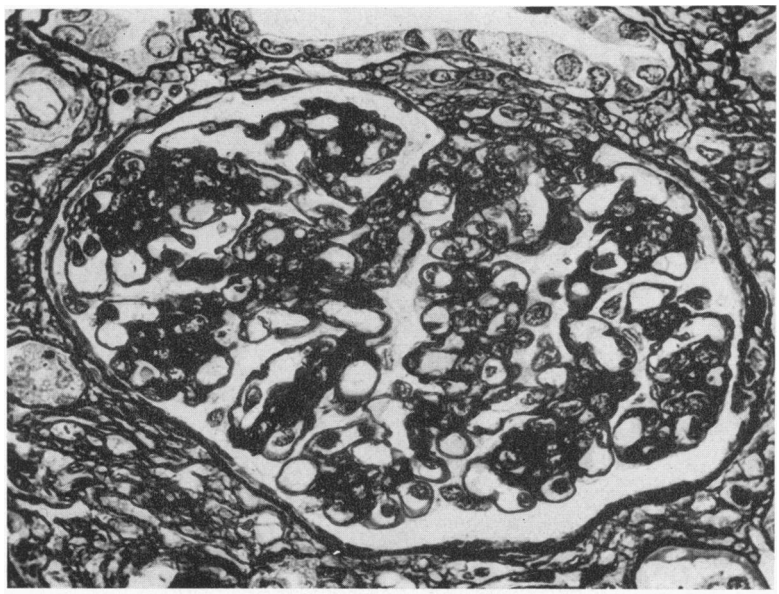
FIG. 4. Nephrotic glomerulonephritis; moderate lesion. This is similar to Figure 3 but is stained with silver methenamine, hematoxylin and eosin. × 490.

FIG. 5. Nephrotic glomerulonephritis; moderate lesion. The glomerulus shows evidence of reactive thickening of the basement membrane. Silver methenamine, hematoxylin and eosin stains. × 450.

FIG. 6. Nephrotic glomerulonephritis; moderate lesion. This glomerulus is jammed with lipid-filled cells, either histiocytic or endothelial. The mesangial cells, surrounded by a mesh of fibromucinous fibrils, are particularly well seen in the lower mid-portion of the field. Silver methenamine, hematoxylin and eosin stains. × 1,020.



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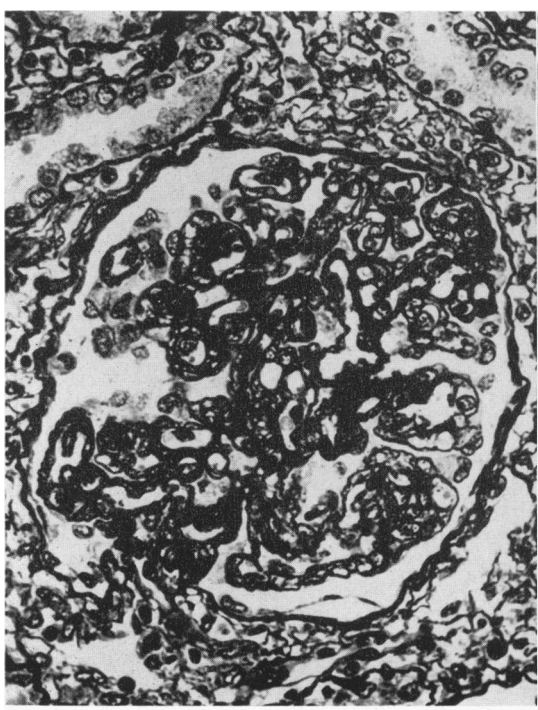

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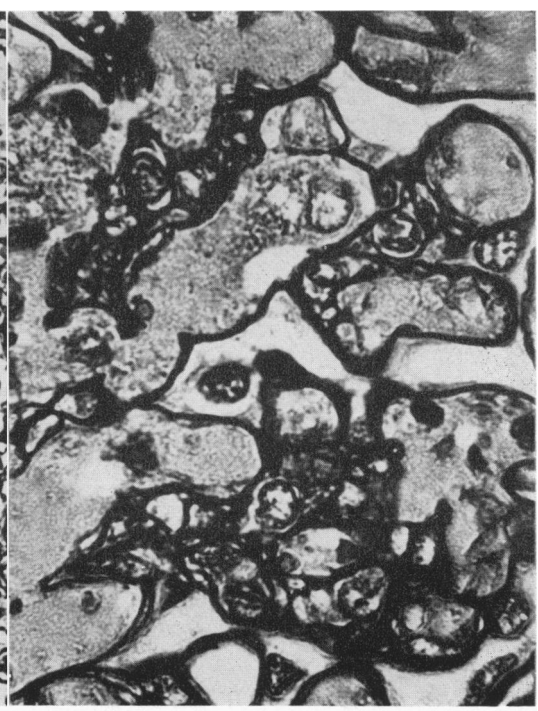

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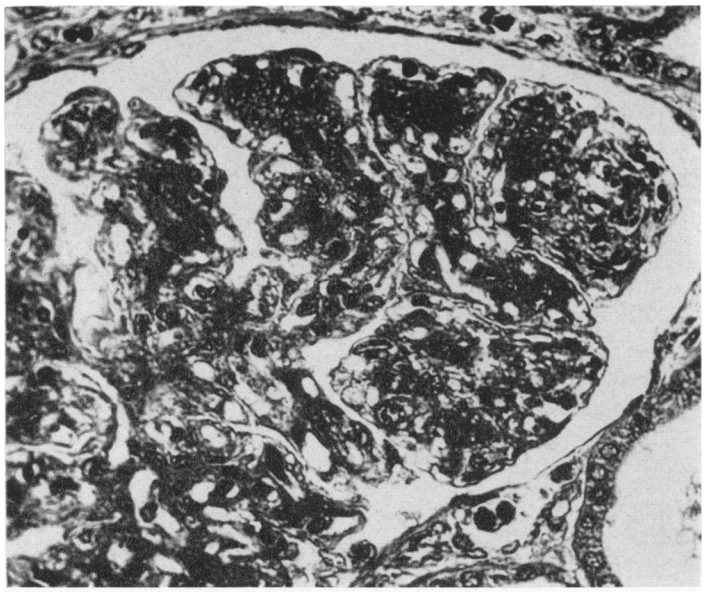
FIG. 7. Nephrotic glomerulonephritis; chronic lobular lesion. Hematoxylin and phloxine stain. × 385.

FIG. 8. Nephrotic glomerulonephritis; chronic lobular lesion. Silver methenamine, hematoxylin and eosin stains. × 440.

FIG. 9. Nephrotic glomerulonephritis; membranous lesion. Of note are the thick capillary walls. Hematoxylin and phloxine stain. × 345.



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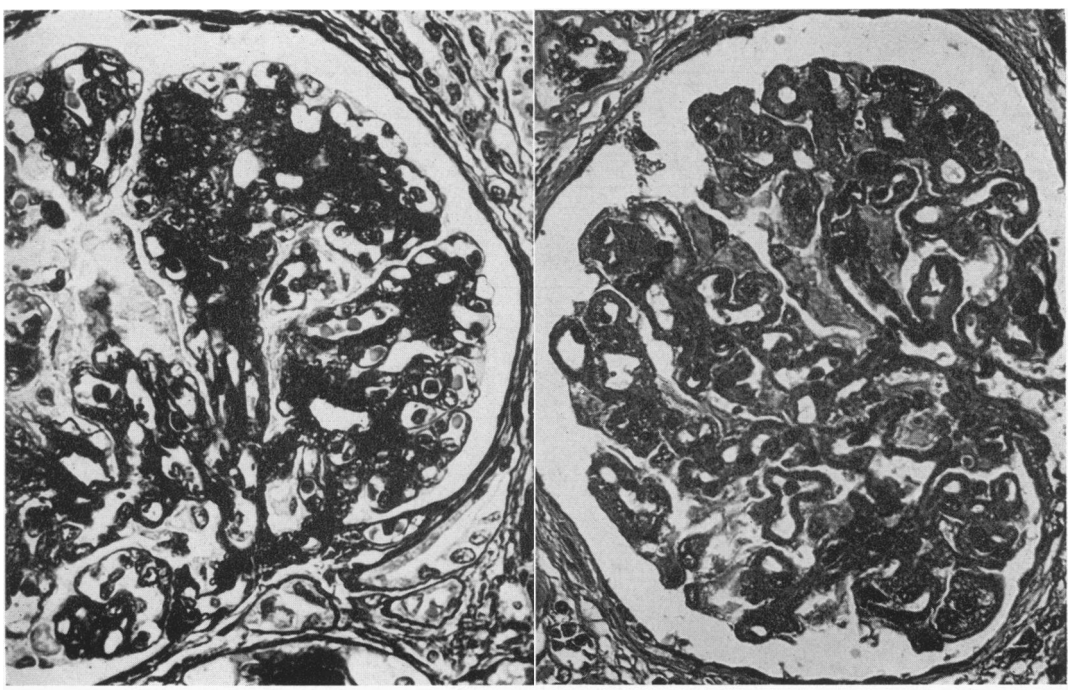

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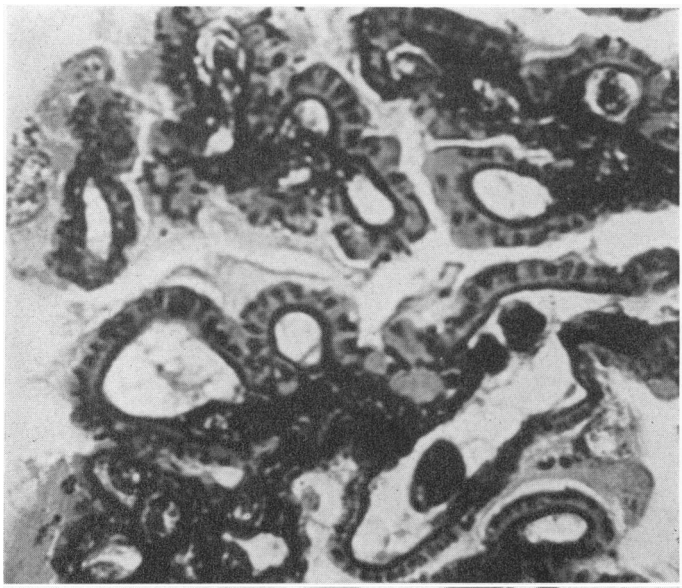
FIG. 10. Nephrotic glomerulonephritis; membranous lesion. The silver-positive clubs separated by hyaline droplets may be seen only fairly well since they are difficult to photograph. Silver methenamine, hematoxylin and phloxine stains. × 1,110.

FIG. 11. Nephrotic glomerulonephritis; membranous lesion. This is the advanced stage of hyalinosis in which only fragments of silver-positive material remain. Silver methenamine, hematoxylin and eosin stains. × 1,110.



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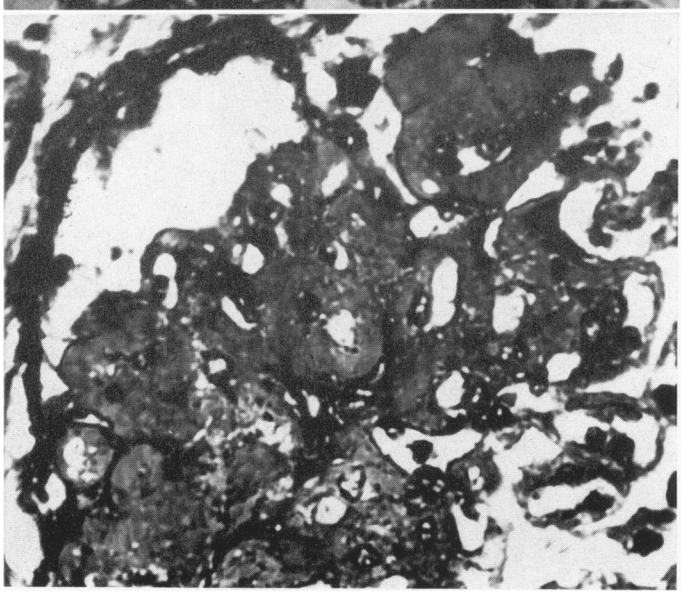

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